新規発根促進剤として有望なエストロジェン活性を持たない5、6-ジクロロインドール-3-酢酸と4-クロロインドール-3-酢酸

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Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council Secretariat
5,6-Dichloroindole-3-acetic acid and 4-chloroindole-3-acetic acid, two potent candidates for new rooting promoters without estrogentic activity

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5,6-Dichloroindole-3-acetic acid (5,6-Cl<sub>2</sub>-IAA, 1) and 4-chloroindole-3-acetic acid (4-Cl-IAA, 2), synthesized from the corresponding chlorinated indole compounds, showed strong rooting-promoting activity in black gram cuttings. 5,6-Cl<sub>2</sub>-IAA was the most potent of all compounds examined. At a concentration of 5×10<sup>-5</sup>M, its activity was 15 times higher than that of 4-(3-indole)butyric acid (IBA), an active ingredient in commercially available rooting promoters. The activity of 4-Cl-IAA was also four times higher than that of IBA at the same concentration. 5,6-Cl<sub>2</sub>-IAA and 4-Cl-IAA had no estrogentic activity as measured using an estrogen receptor binding assay. © Pesticide Science Society of Japan

Keywords: rooting promoter, 5,6-dichloroindole-3-acetic acid, 4-chloroindole-3-acetic acid, estrogentic activity, endocrine-disrupting activity.

Introduction

Many compounds from a variety of chemical categories, including pesticides, food additives, and environmental contaminants, have adverse effects on aquatic animals and mammals, and by extension most likely on humans. Polychlorinated dibenzo-p-dioxins and biphenyls, plasticizers (bis(2-ethylhexyl)phthalate, dibutylphthalate, etc.), pesticides (DDT, atrazine, benomyl, etc.), food additives (di-n-butylhydroxyanisole, morin, etc.), and resin materials (bisphenol A, alkylphenols) are well-known endocrine disruptors.1-7) Many pesticides, in particular chlorinated insecticides, exert endocrine-disrupting activity.1-9

We have been developing new rooting promoters for the mass production of saplings of important, useful and rare trees for the purposes of reforestation and preservation of endangered plant species. Seradix (Oxyberon, Hormadin) and Rooton (NAD, Transplanto), well-known commercially available rooting promoters, are used to make saplings from cuttings of flowers and ornamental plants as well as from trees; however, there are many cases in which it is difficult if not impossible to induce plant roots despite using the above rooting promoters at varying concentrations under diverse conditions. Indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA), active ingredients of the rooting promoters, were natural and synthetic auxins, respectively, but 4-chloro-2-methylphenoxyacetic acid, a synthetic chlorine-containing auxin, has been reported to have an endocrine-disrupting effect,9 however, estrogentic activities of IBA and NAD have not been reported. For these reasons, it has been hoped for the development of potent new rooting promoters without endocrine-disrupting activity, so it is important that the estrogentic activity of newly developed rooting promoters with auxin activities should be checked, as well as their toxicities, before they are made commercially available.

The synthetic 5,6-dichloroindole-3-acetic acid (5,6-Cl<sub>2</sub>-IAA, 1) is the most active of the known natural and synthetic auxins, as determined by Avena coleoptile elongation, inhibition of Chinese cabbage hypocotyl growth, mung bean hypocotyl swelling, and lateral root formation.11) 4-Chloroindole-3-acetic acid (4-Cl-IAA, 2) and its ester also have strong auxin activities and significantly boost the root formation of Serissa japonica cuttings.11) 4-Cl-IAA, the most active natural auxin, is isolated from immature seeds of plants belonging to the Viceae tribe,12-22 some of which are consumed daily by humans, and from immature and mature scotch pine seeds. 5,6-Cl<sub>2</sub>-IAA and 4-Cl-IAA will be potent candidates as active ingredients of new rooting promoters. We report here that these two compounds are highly potent candidates for new rooting promoters without estrogentic activity (Fig. 1).

![Chemical structures of 5,6-Cl<sub>2</sub>-IAA and 4-Cl-IAA.](Fig_1.png)
Materials and Methods

Chemicals. 4-Cl-IAA was synthesized from 2-chloro-6-nitrotoluene using the procedure reported previously by Katayama.\(^{11}\) 5,6-Cl\(_2\)-IAA was newly synthesized from 5,6-dichloroindole in a similar manner. 4-(3-Indole)butyric acid (IBA) and IAA were purchased from Kanto Kagaku Co., Ltd. (Tokyo, Japan) and Merck Co., respectively.

Plant Materials. Black gram (Vigna mungo (L.) Hepper; Sakata Seed Co., Japan) seeds, which had been stored at 5°C, were used in the subsequent rooting-promoting activity test. Black gram seeds were sowed into Akadama soil (Fujimi Gardening Materials Co., Shizuoka, Japan) and grown at 25°C in an incubator (Shimadzu BEC-II-250HUP) under a 16-h light (7,000 lux)/8-h dark cycle for 9 days.

Rooting-promoting activity test with black gram cuttings. Seven-centimeter cuttings with purplish-red hypocotyls were taken from the tops of young plants about 9–10 cm tall. The hypocotyls of cuttings were first soaked in running water and then in sample solution. An ethanol solution (250 μl) of the sample (2×10\(^{-2}\) M) was placed in a deep Petri dish (Φ6×6 cm) and the solvent was evaporated in vacuo in a desiccator at 5°C. The aqueous solution in the dish was sonicated in hot water (ca. 70°C) for 5 min, and then the solution was cooled to room temperature. Ten 2-leafed healthy cuttings with purplish-red hypocotyls were soaked in the cooled aqueous solution for 3 h. The soaked cuttings were washed thoroughly with running water, placed into a deep Petri dish (Φ6×6 cm) with 100 ml water via a hole (Φ18 mm) in the colorless plastic lid, and kept in an incubator (Shimadzu BITEC-400L) under a 16-h light (2,300 lux)/8-h dark cycle for two weeks. The water was changed every two days. After incubation, the number of induced roots was counted and their dry weights were measured after air-drying at 25°C for two weeks. As a control, cuttings were soaked in an aqueous solution containing the spreading agent without the test compound. Duplicate bioassays were conducted five times.

Estrogen receptor binding assay. An estrogen-R(α) competitor screening kit was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). 17β-Estradiol and bisphenol A used as standard samples were also purchased from Wako Pure Chemical Industries Ltd. Fluorescence intensities were measured with a Perkin-Elmer fluorescence microplate reader (Perkin-Elmer).

The binding activity of the chlorinated auxins to estrogen receptors was measured twice with a commercially available estrogen-R(α) competitor screening kit. Six microtiter plates of a dimethyl sulfoxide (DMSO) solution of the sample were mixed with 114 μl of the reaction solution containing fluorescence-labeled estradiol in a test tube. One hundred microliters of the mixture were pipetted into each ER(α)-coated well of a microplate. As controls, 95 μl of the reaction solution and 5 μl DMSO were added to estrogen-R(α)-coated or non-coated wells. All mixtures were incubated at 25°C in an incubator for 2 h. The microplates were washed with 200 μl washing solution and residual solution was completely drained from the well plates by inverting them before 100 μl test solution was added to each well. The fluorescence intensity of each sample was measured at an excitation wavelength of 485 nm and emission wavelength of 535 nm by a fluorescence microplate reader, and the percent value of relative fluorescence units (RFU) was calculated.

Results and Discussion

Among 10 dichloroindole-3-acyclic acids, 5,6-Cl\(_2\)-IAA has been shown to have the strongest activity in terms of elongating the Avena coleoptile, inhibiting Chinese cabbage hypocotyl growth, and inducing hypocotyl swelling and lateral root formation of intact black gram.\(^{10}\) 5,6-Cl\(_2\)-IAA induced many short lateral roots on swollen and growth-inhibited hypocotyls at 1×10\(^{-6}\)–3×10\(^{-5}\) M in 3 days, but did not induce roots by the treatment of intact plants at the same concentration for 3 hr. In this study, 5,6-Cl\(_2\)-IAA was the most effective substance for promoting root formation in black gram cuttings by treatment for 3 hr (Table 1). At a concentration of 5×10\(^{-5}\) M, its induced root number was 15 times more than that of IBA, the active ingredient in commercially available rooting agents, such as Seradix, Hormodin and Oxyberon. Black gram hypocotyl, with its large number of roots, looked like a test tube brush (Fig. 2). Further, many root primordia were induced on the upper portion of hypocotyls which had not been soaked in sample solution but might have grown roots if they had. In addition to the enhancing effect of 5,6-Cl\(_2\)-IAA on root formation, 5,6-Cl\(_2\)-IAA also had strong rooting-promoting activity in that the induced root number was four times more than that of IBA at the same concentration. The dry weight of roots induced by 5,6-Cl\(_2\)-IAA and 4-Cl-IAA was 1.8 times that of those induced by IBA. The smaller increase in dry root weight relative to rooting activity is shown to have the strongest activity in terms of elongating the Avena coleoptile, inhibiting Chinese cabbage hypocotyl growth, and inducing hypocotyl swelling and lateral root formation of intact black gram.\(^{10}\) 5,6-Cl\(_2\)-IAA induced many short lateral roots on swollen and growth-inhibited hypocotyls at 1×10\(^{-6}\)–3×10\(^{-5}\) M in 3 days, but did not induce roots by the treatment of intact plants at the same concentration for 3 hr. In this study, 5,6-Cl\(_2\)-IAA was the most effective substance for promoting root formation in black gram cuttings by treatment for 3 hr (Table 1). At a concentration of 5×10\(^{-5}\) M, its induced root number was 15 times more than that of IBA, the active ingredient in commercially available rooting agents, such as Seradix, Hormodin and Oxyberon. Black gram hypocotyl, with its large number of roots, looked like a test tube brush (Fig. 2). Further, many root primordia were induced on the upper portion of hypocotyls which had not been soaked in sample solution but might have grown roots if they had. In addition to the efficacy related to direct enhancement of root formation, 5,6-Cl\(_2\)-IAA may effectively permeate the hypocotyl and/or travel rapidly once inside the plant. 4-Cl-IAA also had strong rooting-promoting activity in that the induced root number was four times more than that of IBA at the same concentration. The dry weight of roots induced by 5,6-Cl\(_2\)-IAA and 4-Cl-IAA was 1.8 times that of those induced by IBA. The smaller increase in dry root weight relative to rooting activity

<table>
<thead>
<tr>
<th>Compound</th>
<th>Root number</th>
<th>Ratio to IBA (%)</th>
<th>Dry weight of roots (mg) /cutting</th>
<th>Ratio to IBA (%)</th>
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<tr>
<td>5,6-Cl(_2)-IAA (1)</td>
<td>157.2±12.5</td>
<td>1526±121</td>
<td>7.4±0.5</td>
<td>176±12</td>
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<tr>
<td>4-Cl-IAA (2)</td>
<td>42.7±3.2</td>
<td>415±31</td>
<td>7.6±0.5</td>
<td>181±12</td>
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<td>IBA(^{10})</td>
<td>10.3±1.2</td>
<td>100±12</td>
<td>4.2±0.4</td>
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<td>IAA(^{9})</td>
<td>6.0±0.4</td>
<td>58±4</td>
<td>3.7±0.3</td>
<td>88±7</td>
</tr>
<tr>
<td>Control</td>
<td>5.7±0.4</td>
<td>55±4</td>
<td>3.3±0.3</td>
<td>79±7</td>
</tr>
</tbody>
</table>

\(^{a}\) All compounds were used at 5×10\(^{-3}\) M. IBA was used as a standard promoter of root formation. Each value is presented as the mean±standard error of the mean, n=5.\(^{b}\) Dry weight of roots was measured after roots were air-dried at 25°C for two weeks. Each value is presented as the mean±standard error of the mean, n=5.\(^{c}\) IBA, 4-(3-indole)butyric acid.\(^{d}\) IAA, indole-3-acyclic acid.
showed that the limited nutrients within the cuttings could be used to form large numbers of roots but that the growth of these roots could not be sustained without additional nutrients in the solution. At lower concentrations of these promoters, the growth and dry weights of roots increased (data not shown). The increased number and dry weight of roots by the promoters is very important for the production of healthy tree saplings during reforestation. 5,6-Cl₂-IAA is the most potent of the known natural and synthetic rooting promoters while 4-Cl-IAA is the most potent of all known natural promoters. These two compounds will be the most appropriate candidates for active ingredients in new strong rooting promoters.²³⁻²⁸

In the estrogen receptor binding assay, neither 5,6-Cl₂-IAA nor 4-Cl-IAA had estrogenic activity at any concentration tested, although many chlorinated pesticides do have endocrine-disrupting activity (Fig. 3).²⁹ On the other hand, bisphenol A, a control compound, showed apparent estrogenic activity at concentrations greater than 1 × 10⁻⁴ M, although this activity was much weaker than that of 17β-estradiol. These results suggest that 5,6-Cl₂-IAA and 4-Cl-IAA as active ingredients of new rooting promoters will have the added benefit of not disrupting endocrine activity. Further, this result demonstrates the safety of edible Viciaeae plants, the immature seeds of which contain 4-Cl-IAA without exception. We are now continuing to improve rooting promoters using these compounds and testing root induction in trees that are usually at least partly resistant to this process.

References
23) M. Mitsuhashi, H. Shibaoka and M. Shimokoriyama: Plant Cell...
ピクル濃度で検出されるものが存在した。

アルキレン架橋ピスイミダクロプリド誘導体の植物浸透移行による殺虫活性

森 勝, 菊池真美, 大野育也, 利部伸三

アルキレン架橋ピスイミダクロプリド誘導体（Bis-IMI）の、シャーレ試験、葉面散布、イネ苗株浸漬処理及び浸透移行性実験条件における殺虫活性を求める。シャーレ試験におけるモリタクアブルムシに対する殺虫活性は架橋の長さによって変動し、ヘプタ（C7）およびオクタメチレン（C8）誘導体が最も高く、10 mg/mLで高い殺虫活性を示した。同濃度の薬剤をキャベツ葉面散布し、11日目にて散布し72時間後に観察したところ、ヘキサメチレンC6およびC7誘導体はほぼ完全にモリタクアブルムシを除殺した。しかし、同様な条件で、トピロウカン（イネ）とコナガ（キャベツ）に対する殺虫試験を行なったところ、両化合物導入チャックは弱かった。一方、イネ苗株の根部浸漬処理及びキャベツ苗株への userManager処理後、3, 7, 11日に散布し72時間後に観察したところ、C7誘導体はトピロウカンとモリタクアブルムシに対して、C6誘導体はトピロウカンに対して高い防除活性を示し、これらの化合物の浸透移行性が確認された。

Bis-IMI誘導体は、分子量、LogP値、水素結合受容性原子数および自由回転結合数において、パラメタスクリーニングシステムによる殺虫性分子のための基準値からはずれている。このような新しい骨格の化合物の殺虫特性は、既存殺虫剤のデータを基にして誘導された判断基準だけでは説明できず、新たな構造分子を含めた考察が必要と思われる。

短 報

土によるテルプメトンとイソプロトロンの吸着：Ca^2+とK^+カチオンの影響

Achouak El Arfaoui, Stephanie Boudesocque, Stephanie Sayen, Emmanuel Guillon

塩（KClとCaCl₂）が、テルプメトンとイソプロトロンの四種の石灰質土壌への吸着に及ぼす影響を、図示実験を用いて調べた。場合によって、Ca^2+とK^+カチオンの存在は土への農薬の吸着量に対し、影響する場合が見られた。

（文責：編集事務局）

新規発根促進剤として有望なエストロジェン活性を持たない5,6-ジクロロインドール-3-酢酸と4-クロロインドール-3-酢酸

片山正人、斎藤隆雄、金山公三

相応のクロル化インドールから合成した5,6-ジクロロインドール-3-酢酸（5,6-Cl₃-IAA）と4-クロロインドール-3-酢酸（4-Cl-IAA）は、モラシマフグに対する強力な発根促進活性を示し、特に、5,6-Cl₃-IAAは最も強力な発根促進活性を示した。5×10⁻⁸ Mの濃度では、5,6-Cl₃-IAAの発根数は市販の発根促進剤の活性成分である4-(3-インドール)酢酸（IBA）の15倍もあり、4-Cl-IAAのそれはIBAの4倍であった。一方、5,6-Cl₃-IAAと4-Cl-IAAのエストロジェン活性をエストロジェンレセプター結合アッセイで調べたところその活性は共に認められなかった。

解説

中国における農薬の使用と残留抑制

Fen Jin, Jing Wang, Hua Shao, Maojun Jin

中国政府の政策計画において農薬の発展問題は依然として重要な課題であり続けている。農薬の発展過程において、農薬の使用量を増加させ農作物の疾病制御に重要な役割を果たしてきた。しかし、最近の研究によれば、農薬の使用量は免疫機能の低下やホルモンの乱れやガンの発症などに関係している可能性が示唆された。したがって、世界で最大の農薬生産国である中国において、農薬の使用と制御は極めて重要な課題である。本論文では、中国における農薬の使用状況を概説するとともに、農薬残留抑制のための対策と提案のいくつかを紹介する。

（文責：編集事務局）